MICROBIOLOGICAL EFFICACY SUMMARY

OVERVIEW

Tristel ORL is a high-level disinfectant foam intended to disinfect cleaned, reusable, non-lumened otorhinolaryngology devices. As a high-level disinfectant, Tristel ORL has been tested for efficacy against mycobacteria, viruses, fungi and bacteria with a contact time of two minutes.

Tristel ORL is proven to be effective against clinically relevant organisms such as *Moraxella catarrhalis*, *Streptococcus pyogenes* (Group A Streptococcus), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Carbapenem-Resistant *Klebsiella pneumoniae*, Extended Spectrum Beta-Lactamase (ESBL) producing *Klebsiella pneumoniae*, *Haemophilus influenza*, Vancomycin-Resistant *Enterococcus faecalis* (VRE), Multi-Drug Resistant *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Candida albicans*, *Aspergillus brasiliensis*, *Aspergillus fumigatus*, Parainfluenza Virus Type 3, Respiratory Syncytial Virus (RSV), Rhinovirus Type 37, SARS-CoV-2, Human Papillomavirus (HPV), Influenza Virus, and Adenovirus Type 5.

To gain marketing authorization, Tristel has submitted data meeting the requirements laid out in the Health Canada guidance '*Safety and Effectiveness Requirements for High-Level Disinfectants and Sterilants for use on Reusable Semi-Critical and Critical Medical Devices (2018).*'

In line with regulatory guidance, Tristel ORL was subjected to a three-tier efficacy testing program:

POTENCY TESTS are conducted to demonstrate the broad spectrum of efficacy of the high-level disinfectant against a list of FDA and Health Canada mandatory test organisms and specific additional efficacy claims.

SIMULATED-USE TESTS are conducted to determine the penetrating capability of a high-level disinfectant used on representative medical devices. A test germicide should be able to kill at least 10^6 inoculated mycobacteria under the recommended contact time.

IN-USE TESTS are conducted to confirm the results of Simulated-Use testing and to evaluate high-level disinfectant performance in clinical use conditions. The aim of testing is to confirm efficacy under strenuous conditions (i.e. persistence and resistance of ambient bioburden, such as biofilms, including "wild type" bacteria, fungi and other unforeseen factors) that could occur in the clinical setting.

POTENCY TESTS

The potency of Tristel ORL has been studied extensively by testing in accordance with the standards applicable to chemical disinfectants marketed in Europe, Australasia, and North America.

Medical device disinfectants approved in the United Kingdom, Europe and Australasia follow the EN 14885:2022 *'Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics'*. This standard stipulates the required test methods, conditions, and performance criteria that must be achieved to claim sporicidal, mycobactericidal, virucidal, fungicidal and bactericidal activity on the disinfectant label.

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In addition, Tristel ORL has been tested using AOAC and ASTM methods specified for potency testing in FDA and Health Canada guidance. The results of these studies demonstrate that Tristel ORL exhibits sporicidal, mycobactericidal, virucidal, fungicidal, and bactericidal activity.

POTENCY TESTS SUMMARY

ORGANISM	TEST METHOD	TEST TYPE	CONDITIONS
SPORES			
Bacillus subtilis	AOAC 966.04	Carrier	No Soil
Clostridium sporogenes			
Clostridium sporogenes	AOAC 966.04 / EN 14561	Carrier	Dirty 1
MYCOBCATERIA			
Mycobacterium terrae	Ascenzi et al., 1987/ASTM E2315	Suspension	Dirty 1
Mycobacterium terrae	EN 14563	Carrier	Dirty 1
Mycobacterium avium			
Mycobacterium bovis	AOAC 965.12	Carrier	Dirty 1
Mycobacterium avium	EN 14348	Suspension	Clean
FUNGI			
Trichophyton interdigitale	AOAC 955.17	Suspension	Dirty 1
Aspergillus fumigatus	AOAC Use Dilution Method	Carrier	Dirty 1
Candida albicans			
Aspergillus brasiliensis	EN 16615	Surface with mechanical action	Clean
Candida albicans			
Candida albicans	EN 13697	Surface	Clean
Aspergillus brasiliensis	EN 14562	Carrier	Clean
Candida albicans			
Candidozyma auris (formerly *Candida auris*)			Dirty 1

Tristel ORL™

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2 min

ORGANISM	TEST METHOD	TEST TYPE	CONDITIONS
VIRUSES			
Poliovirus Type 1	ASTM E1053	Surface	Dirty 1
Herpes Simplex Virus (HSV) Type 1			
Adenovirus Type 5			
Influenza Virus			
Human Norovirus Surrogate (Feline Calicivirus)			
Human Hepatitis B Virus Surrogate (Duck Hepatitis B Virus)			
Human Immunodeficiency Virus (HIV) Type 1			
Parainfluenza Virus Type 3			
Respiratory Syncytial Virus (RSV)			
Rhinovirus Type 37			
Poliovirus Type 1	EN 14476	Suspension	Clean
Adenovirus Type 5			
Murine Norovirus			
SARS-CoV-2			
Poliovirus Type 1	DVV & RKI	Suspension	Dirty 3
Adenovirus Type 5			
Murine Norovirus			
Vaccinia Virus			
Human Papillomavirus (Using Polyoma virus SV40 as a Surrogate)			
BACTERIA			
Staphylococcus aureus	AOAC 955.15	Carrier	Dirty 1
Pseudomonas aeruginosa	AOAC 964.02		

MICROBIOLOGICAL EFFICACY SUMMARY

ORGANISM	TEST METHOD	TEST TYPE	CONDITIONS
BACTERIA			
Salmonella enterica	AOAC 955.14	Carrier	Dirty 1
Moraxella catarrhalis	AOAC Use Dilution Method	Carrier	Dirty 1
Streptococcus pyogenes			
Haemophilus influenzae			
Vancomycin-Resistant *Enterococcus faecalis* (VRE)			
Multi-Drug Resistant *Streptococcus pneumoniae*			
Staphylococcus epidermidis			
Streptococcus agalacticae			
Neisseria gonorrhoeae			
Chlamydia trachoatis	Bespoke ASTM E1053	Surface	Dirty 1
Staphylococcus aureus	EN 14561	Carrier	Clean
Enterococcus hirae			
Pseudomonas aeruginosa			
Carbapenem-Resistant Enterobacteriaceae (CRE) *Klebsiella pneumoniae*			
Vancomycin-Resistant *Enterococcus faecium* (VRE)			
Methicillin-Resistant *Staphylococcus aureus* (MRSA)			Dirty 1
Multidrug-Resistant *Acinetobacter baumannii* (MDRAB)			
Extended Spectrum Beta-Lactamase (ESBL) producing *Klebsiella pneumoniae*			
Staphylococcus aureus	EN 16615	Surface with mechanical action	Clean
Enterococcus hirae			
Pseudomonas aeruginosa			
Staphylococcus aureus	EN 13697	Surface	Clean

ORGANISM	TEST METHOD	TEST TYPE	CONDITIONS
BACTERIA			
Enterococcus hirae	EN 13697	Surface	Clean
Escherichia coli			
Pseudomonas aeruginosa			

KEY

Clean	0.3 g/l Bovine albumin
Dirty 1	5% Blood Serum
Dirty 2	3g/l Bovine albumin + 3ml Blood erythrocytes
Dirty 3	10% Blood Serum

SIMULATED-USE TESTS

The otorhinolaryngology or ear, nose, and throat (ENT) devices evaluated include a **flexible fibreoptic rhino-laryngoscope** and a **portable video laryngoscope**. The flexible fibreoptic rhino-laryngoscope is designed for minimally invasive examination of the upper airway, including the nasal passages, pharynx, and larynx. The portable video laryngoscope is used for endotracheal intubation and enables visualisation of the vocal cords via an integrated camera, thereby improving first-pass success rates, particularly in patients with difficult airways.

These ENT devices may contact mucous membranes or non-intact skin during clinical practice and therefore requires high-level disinfection in accordance with the Spaulding classification system (Spaulding, 1968).

Tristel worked with leading ENT device manufacturers to identify representative ENT devices for simulated-use testing. The criteria for device selections were set as:

- Worst case devices with difficult to penetrate design features like ridges, crevices or indentations,
- Different shapes and materials of construction,
- Device models used in North America.

The microbial challenge applied to the devices consisted of at least 10^6 Colony Forming Units (CFU) of *Mycobacterium terrae*. To further challenge the germicidal efficacy of Tristel ORL, organic and inorganic substances were incorporated into the test inoculum. 5% bovine serum albumin (BSA) and AOAC hard water at 400ppm calcium carbonate were used in testing.

Patient contacting portions of the devices were immersed in the microbial and inorganic/organic inoculum. This technique ensures a complete and thorough microbial challenge had been applied to each device.

Following immersion, the devices were dried for at least one hour before performing the Tristel ORL high-level disinfection procedure according to the Tristel ORL user instructions.

After high-level disinfection the devices were submerged in the recovery/neutralization fluid to recover viable microorganisms remaining on the surface. The recovery/neutralization fluid was filtered through membrane filters and placed on Middlebrook Agar (MBA) plates. All plates were incubated for 21 days at 37 ± 2 °C.

Controls and validations included positive (inoculum and growth), negative, neutralization validation, low-level inoculum and media sterility controls. Each test device was tested in triplicate to provide statistical significance in the test results.

SIMULATED-USE TESTS SUMMARY

According to FDA and Health Canada guidance, Tristel ORL demonstrated high-level disinfection by achieving at least a 6 $\log_{10}$ (10^6) reduction in inoculated mycobacteria within a contact time of two minutes.

DEVICE MANUFACTURER	DEVICE TYPE	MODEL	RESULTS (AVERAGE $\log_{10}$ REDUCTION)
MYCOBACTERIUM TERRAE			
Karl-Storz	Rhino-Laryngoscope	11101RP2	6.4
Medtronic	Video Laryngoscope	McGRATH™ MAC, REF 301-000-000	8.0

Efficacy was demonstrated in a study using infectious HPV types 16 and 18 on an endocavitary ultrasound probe, achieving the required 4 $\log_{10}$ (10^4) reduction in viral load.

DEVICE MANUFACTURER	DEVICE TYPE	MODEL	RESULTS (AVERAGE $\log_{10}$ REDUCTION)
HUMAN PAPILLOMAVIRUS TYPES 16 & 18			
Siemens Healthineers	Endocavitary ultrasound probe	Acuson EC9-4	4.9 $\log_{10}$ reduction in HPV Type 16
			4.1 $\log_{10}$ reduction in HPV Type 18

IN-USE TESTS

STUDY DESIGN AND TEST DEVICES

Pentax flexible nasendoscopes (Model: VNL-1070STK and VLS1070STK) were selected as the test device for the in-use study. These devices are representative of ENT devices routinely used for ENT examinations and procedures. The devices incorporate design features that may impede effective cleaning and disinfectant penetration, including indentations and ridges, and therefore represent a worst-case challenge for reprocessing.

Two microbial endpoints were assessed:

- Bacterial Recovery
- Fungal Recovery

CLINICAL USE AND DISINFECTION PROCEDURE

The ENT devices were used in routine clinical practice for patient procedures. Following patient use, clinic personnel disinfected the devices using Tristel ORL, applying three doses rather than the labelled four-dose application. Tristel staff did not participate in the device decontamination process.

After completion of the two-minute contact time, the devices were placed on a sterile surface for sampling.

SAMPLING AND RECOVERY METHOD

Sampling was performed using sterile swabs. Each swab was dipped into 10 ml of recovery fluid and used to thoroughly sample one of the following areas:

- Device tip
- Bending rubber
- Insertion tube

Note: Control swabs were taken from four sides of the device handle.

For each sampling area, two swabs were used and recovered into a single 10 ml volume of recovery fluid. Therefore, per device, a total of six swabs were recovered into the same 10 ml of recovery fluid.

The recovery fluid tubes containing the swabs were placed in an ice chest with ice packs or stored in a hospital-provided refrigerator. Samples were then transported to the microbiology laboratory for plating and analysis.

CONTROLS AND MICROBIOLOGICAL ANALYSIS

Two devices were sampled after clinical patient use and before cleaning and disinfection to establish a baseline of wild-type bacteria or fungi present on the patient contaminated devices, these are the positive control devices.

To differentiate under non-selective conditions, one set of 3 devices were extracted and the extraction media plated under conditions favourable to bacterial growth, Trypticase Soy Agar (TSA) and incubation at 36 ± 1 °C. An independent set of 3 devices were extracted and plated under conditions favourable to fungal growth, Potato Dextrose Agar (PDA) and incubation at 30 ± 2 °C.

POST-SAMPLING DEVICE HANDLING

After sample collection, the devices were returned to clinic personnel for reprocessing in accordance with the clinic's standard process before its return to use on a patient.

IN-USE TESTS SUMMARY

Results of testing demonstrates that Tristel ORL meets the labeling claims for high-level disinfection under in-use conditions.

SAMPLE/TREATMENT	NUMBER OF SAMPLES TESTED	BACTERIA RECOVERED	FUNGI RECOVERED	CONCLUSION ON MICROBIAL SURVIVAL
Baseline Positive Controls (Not Cleaned or Disinfected)	2	Microorganisms recovered	Microorganisms recovered	Confirmed pre-disinfection contamination
Test Samples (Cleaned and Disinfected with Tristel ORL)	6	No bacteria recovered	No fungi recovered	No microbial survival